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Research Article

TSH Receptor Antibodies as Measured in the Thyroid Stimulating Immunoglobulin (TSI) Reporter Bioassay Thyretain are not Detected in Patients with Euthyroid Graves' Disease

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Abstract

Background: The ophthalmopathy associated with Graves' hyperthyroidism is most likely a T-cell mediated disorder, although most investigators believe that autoantobodies directed against the TSH Receptor (TSH-R) expressed on the surface of the orbital fibroblasts and pre-adipocytes are the main driver of the orbital reactions. Cases of ophthalmopathy without TSH-R binding antibodies and not closely associated with Thyroid Stimulating Immunoglobulin's (TSI), challenge the notion that ophthalmopathy is perpetrated by TSI circulating in peripheral blood and TSI producing plasma cells resident in the orbital tissues. One way to address a possible role of TSH-R antibodies in the development of ophthalmopathy is to study patients with so-called "Euthyroid Graves' eye Disease (EGD)", who have **a similar** eye disorder but with normal thyroid function and no features of thyroid autoimmunity during long term follow up.

Methods: The cell-based reporter bioassay Thyretain ™ was used to assess TSI in serum of the patients (n=49) and control subjects (n=20); The patient groups; EGD (n=12), of whom 6 were first tested early in the course of their eye disorder (< 6 months) and 7 tested ≥ 6 months after the onset of eye symptoms, Graves' disease with (n=13), and without (n=12), ophthalmopathy and euthyroid relatives from a previously studied family having a high prevalence of thyroid autoimmunity and ophthalmopathy (WH-Fam n=12). Thyretain ™-TSI results were expressed as per cent of the sample to reference ratio (SRR %), a positive test being taken as an SRR% of > 140%.

Results: Overall, the patients with EGD had mild to moderate eye disease as determined from NOSPECS classes and scores. Only 3 of the 12 patients (25%) initially diagnosed with EGD tested TSI positive in one or more serum samples and all 3 had converted to Graves hyperthyroidism at the time of the positive test or soon after. Among the remaining 9 EGD patients who tested TSI negative, none developed thyroidism autoimmunity during follow up visits 6 months to 10 years after initial diagnosis. Normal subjects and euthyroid relatives from WH-Fam all tested TSI negative whereas 9 out of 12 (75%) Graves' Hyperthyroidism (GH) and 11 out of 13 (86%) Graves' Ophthalmopathy (GO) patients tested TSI positive.

Conclusion: The finding of positive TSI among patients with GH and GO, but not among patients with EGD with the exception of the 3 patients who developed Graves' hyperthyroidism, strongly suggests that TSH-R antibodies cannot play a major role in the pathogenesis of what is best called endocrine or autoimmune ophthalmopathy.

Keywords: Ophthalmopathy; Thyroid autoimmunity; TSH receptor antibodies; Euthyroid Graves' disease; Thyretain ™ TSI reporter bioassay

Introduction

Euthyroid Graves' disease (EGD) is an autoimmune condition of the eye and orbital tissues in the absence of thyroid dysfunction and autoimmunity and is presumed to be the same eye disorder that is associated with Graves hyperthyroidism, where it is known as "Graves ophthalmopathy (GO)". Some of these patients do develop Graves' hyperthyroidism or Hashimoto thyroiditis on long term follow up [1] but, in a more recent study [2], none of 7 patients with EGD developed any evidence of thyroid dysfunction or autoimmunity during up to 11 years of follow up. In patients with EGD as defined, thyroid peroxidase (TPO), thyroglobulin (TG) and TSH receptor (TSH-R) antibodies measured as TSH-R binding immunoglobulins (TBII) (e.g. TRAb as measured in a popular commercial TBII assay), and

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as thyroid-stimulating immunoglobulin (TSI) measured in a cAMP reporter bioassay, were always negative [2]. The pathogenesis of GO, which is presumed to be the same eye disorder but associated with hyperthyroidism, is best explained by the action of T-lymphocytes and antibodies that target autoantigens shared between the thyroid and orbit muscle tissue, such as the TSH-R expressed on the orbital pre-adipocytes and fibroblasts [3-6], the main candidate antigen. However, the TSH-R is expressed in non-thyroid tissues as well and anti-TSH-R antibodies are not detected in all patients with GO at the time of diagnosis [7], nor in patients with Hashimoto thyroiditis with mainly upper eyelid signs and symptoms [8,9]. For this reason, the conspicuous absence of TSH-R antibodies upon multiple visits of patients with EGD implies that anti-TSH-R antibodies are neither necessary nor sufficient for the pathogenesis of ophthalmopathy associated with thyroid autoimmunity.

TSI by Thyretain ™ -reporter bioassay (Thyretain-TSI) were previously shown to be functional indicators of the activity and severity of GO; furthermore, the combination of negative TRAb and positive Thyretain-TSI is closely associated with ophthalmopathy (Lytton *et al* [10-12] while patients testing positive for TRAb and negative for Thyretain-TSI had Graves hyperthyroidism without orbitopathy [12]. In the present study we have used the Thyretain ™ bioassay to assess the TSI levels in sera from 12 patients with EGD, including several seen on repeated visits within a few months of the onset of symptoms. The TSI levels of these EGD patients were compared with normal controls and with three other patient groups; hyperthyroid Graves' disease patients with and without ophthalmopathy and 12 euthyroid relatives from a single family with a high prevalence of thyroid autoimmunity.

Clinical Subjects and Methods

Clinical subjects

We studied sera from; 1) 12 patients with euthyroid Graves' disease, 8 females and 4 males aged 43-70 (mean age 56 years). Multiple serum samples from 6 of the patients and single samples from 6 patients were tested in single Thyretain TSI reporter bioassays. All 12 patients were diagnosed as "euthyroid Graves' disease" at the time of the initial blood sample although it was later shown that 3 of the patients (nos. 1, 2 and 3, Table 1) subsequently converted to Graves hyperthyroidism (2 patients) or had developed Graves disease earlier (1 patient).

2) 12 euthyroid members of a single family with a high prevalence of Graves disease (WH-Fam) [13] namely, 8 females and 4 males, aged 13 -76 (mean age 40 years).

3) 25 patients with Graves' disease, including 3 of the probands from WH-Fam with Graves' disease, 20 females and 5 males aged 16 – 77 (mean age 51 years) of whom 10 females and 3 males aged 38 - 77 (mean age 57 years) had ophthalmopathy (GO). Blood samples were taken at various times in these patients, usually soon after diagnosis and at various times during anti thyroid drug therapy. Most patients had been TSH-R antibody positive in the TRAb and cAMP based TSI assays on at least one occasion.

4) 20 normal subjects comprising; 15 males aged < 30 whose sera had been previously used to develop a reference range for an ELISA to measure CASQ1 and collagen XIII antibodies and 5 other subjects, 4 females and one male, aged 28 – 50 (mean age 32 years).

The diagnosis of the various thyroid disorders was made according to conventional clinical parameters and confirmed by serum free T4 (fT4), free T3 (fT3) and TSH measurements and from anti - TG and anti - TPO antibody tests and, in many cases, real time thyroid ultrasonography. Local Ethical Committee approval was received for this retrospective study and informed consent was not required.

Eye assessment

The ophthalmopathy was assessed as; i) Nunery type 1 (without restrictive myopathy) or type 2 (with restrictive myopathy) [14] ii) a modified Clinical Activity Score (CAS) (0-12) of Mourits *et al.* [15] which is a measure of disease activity iii) Werner's NOSPECS classes [16] and a NOSPECS score (0-18) derived from scores of 0-3 for each of the main features namely, inflammatory changes, upper eyelid retraction (UER), ocular myopathy, proptosis, corneal involvement and optic nerve compression, and iv) upper eyelid margin-reflex distance (MRD) which is the distance between the centre of the pupillary light reflex and the upper eyelid margin with the eye in primary gaze, as a measure of eyelid retraction; an MRD of > 5mm is taken as significant UER. The degree of proptosis (mm) was measured using a Hertel exophthalmometer where a positive reading was defined as > 18mm in either eye or > 2 mm difference between the eyes. For the purpose of the study "ophthalmopathy" was taken as a NOSPECS class ≥ 2, regardless of the CAS.

Thyretain ™ TSI reporter bioassay

The Thyretain ™-TSI cAMP luciferase reporter bioassay described previously [10-12] was used to assess TSI. Briefly, test serum samples and four controls, consisting of reference standard bovine TSH (bTSH), normal serum, positive TSI serum and cells alone, were tested in triplicate. Results were expressed as per cent of the sample to reference ratio (SRR%). The cut-off of the Thyretain-TSI reporter % of the sample to reference ration (SRR%) was historically established as 2SD above the reference luminescence, which is set to SRR 100% for each plate, a positive test being taken as an SRR% of > 140%.

Other tests

Plasma fT4, fT4 and TSH levels and serum TPO and TG antibodies were measured by Barratt and Smith Pathology, Sydney, Australia, according to the manufacturers' instructions.

Statistical analysis

Differences in the TSI levels between the various Graves disease patient groups and the control subjects were assessed using the Mann-Whitney test for non parametric data; a p value of <0.05 was taken as statistical significance.

Results

We used a new cell-based bioassay for Thyretain-TSI that has been shown to measure those TSH-R antibodies that are functional indicators of the existence; activity and severity of Graves' ophthalmopathy (GO) in 12 patients with EGD. All 12 patients were referred to the Thyroid Clinics with the diagnosis of EGD, for eye muscle antibody testing. Demographics, thyroid function and thyroid ultrasound findings at the first visit in the patients with EGD are summarised in Table 1 and eye findings, smoking status and treatment given are summarised in Table 2. In Table 3 are shown

Tab1e 1: Demographics, thyroid function and thyroid ultrasound findings at the first visit in 12 patients with Euthyroid Graves' disease.

Patient number	Age/gender	Ethnicity	Thyroid function[1]		Thyroid ultrasound	Histological assessment[2]
			fT4	TSH		
1	55/F	Caucasian	12.3	0.74	Normal	NP[3]
2	43/M	Caucasian	Normal	Normal	NP	NP
3	70/F	Caucasian	Normal	Normal	NP	NP
4	54/F	Caucasian	14	0.42	Normal	NP
5	50/F	Asian	9.9	0.84	Not performed	NP
6	60/F	Asian	14	0.69	One small nodule else normal	NP
7	63/F	Caucasian	12.6	1.1	Normal	NP
8	53/F	Malay	16	1.91	One large nodule, one small nodule else normal	Follicular cell adenoma, no small lymphocytes on FNAB or at thyroidectomy
9	49/F	Caucasian	13	0.97	Several big nodules and overall colloid features	Colloid goitre, no lymphocytes at thyroidectomy
10	51/M	Caucasian	9.9	Normal	Small nodules	NP
11	63/M	Caucasian	13.7	0.63	Normal	NP
12	65/M	Caucasian	15.2	0.88	NP	NP

[1] fT_4 = Free T4, TSH = thyroid stimulating hormone
[2] Determined from fine needle aspiration (FNA) biopsy (FNAB) (patient no. 8) or histological assessment following thyroidectomy (patient no. 8, 9)
[3] NP = Not performed

Table 2: Eye findings, smoking status, orbital CT findings and treatment in 12 patients with Euthyroid Graves' disease at the first visit.

Patient number	Smokes	Eye Signs				Orbital CT findings		Treatment
		NOSPECS Class [3]	NOSPECS score (0-18)	CAS[1]	Nunery Type[4]	UER[2]		
1	No	4	3	1	2	Yes	EOM [5] volumes increased	Prednisolone 7.5 mg
2	No	4	4	4	2	No	NP [6]	Nil
3	No	2	2	3	1	No	NP	LT4 @ time of second blood test
4	Yes	2	2	3	1	No	NP	Nil
5	No	3	6	5	1	Yes	Normal EOM	Prednisolone in the past
6	No	2	3	3	1	No	Normal on two occasions 1 yr. apart	Prednisolone (50mg/day) + steroid eye drops
7	No	4	4	4	2	Yes	Enlarged EOM[5]	Nil
8	No	4	3	4	2	No	Enlarged EOM	Prednisolone 25 mg
9	No	3	3	4	1	No	Minimal EOM	Nil
10	No	4	3	4	2	No	Enlarged EOM on left only	Nil
11	No	4	5	6	2	Yes	EOM volumes increased	Nil
12	No	4	2	1	2	No	Enlarged left inf. rectus muscle only	Nil

[1]CAS = ativity score of *Mourits et al* [15]
[2]UER = upper eyelid retraction
[3]NOSPECS classes of Werner [16]
[4]Nunery types 1 (ophthalmopathy without ocular myopathy) and 2 (with ocular myopathy) [14]
[5]EOM = extra ocular muscles
[6] NP = not performed

serum thyroid antibodies, other autoantibodies and personal and family history of thyroid and other autoimmune disorders in these patients. Serum T4, T3 and TSH levels were always normal in these patients, and serum TPO and TG antibodies (measured in ELISA) were always negative (Table 3). TSH-R antibodies measured in the TRAb assay were negative in all patients except on one occasion only in one patient (results not shown) which corresponded to a positive Thyretain-TSI test (see below), while thyroid stimulating antibodies measured in a conventional cAMP based immunoassay were negative in all patients tested on all occasions except in patient no. 4 who had a positive test – but negative TRAb and Thyretain-TSI – on one occasion only (results not shown). Where measured, antinuclear antibody tests were negative in all but one patient. One other patient had a positive gastric parietal cell (GPC) antibody test and 3 had positive tests for the flavoprotein (Fp) (the "64 kDa protein") antigen.

NOSPECS classes at the first visit ranged from 2-4, mean 3.4 and NOSPECS scores ranged from 2-6, mean 3.3 (Table 2). Clinical Activity Scores (CAS) ranged from 1-6, mean 3.5. Seven patients had eye muscle dysfunction (Nunery 2) (Table 2). The overall severity and activity of the eye disorder in the 12 patients with EGD can be classified as mild to moderately severe. An example of a patient with

Table 3: Thyroid antibodies, other autoantibodies and personal and family history of thyroid and other autoimmune disorders in 12 patients with Euthyroid Graves' disease at the first visit.

Patient Number	Thyroid antibodies[1]		Other autoantibodies[2]		Personal history of thyroid or other autoimmunity	Known family of thyroid or other autoimmunity
	TPO	TG	ANA	Other		
1	<20	<20	Nag	NT[3]	Nil	"Thyroid disease"
2	<20	<20	NT	Fp	Not known	Nil
3	<20	<20	NT	NT	Not known	Nil
4	<20	<20	NT	NT	Not known	Nil
5	<20	<20	Nag	NT	Nil	Rheumatoid Arthritis
6	<20	<20	Nag	GPC	Vitiligo	Nil
7	<20	<20	Nag	Fp	Nil	Hashimoto's thyroiditis
8	<20	<20	Nag	Fp	Nil	Nil
9	<20	<20	Nag	NT	Nil	Nil
10	<20	<20	NT	NT	Nil	Nil
11	<20	<20	Pos 1/80	NT	Not known	Nil
12	<20	<20	NT	NT	Not known	Nil

[1]Thyroid antibodies were measured in a commercial ELISA, TPO = thyroid peroxidase (cut off titre 20), TG = thyroglobulin (cut off titre 20)
[2]ANA = anti nuclear antibody, Fp = flavoprotein (the 64 kDa protein), GPC = gastric parietal cell
[3]NT = not tested

EGD and severe congestive changes but no exophthalmos or eye muscle dysfunction is shown in Figure 1. The time between onset of symptoms and the first TSI test varied from 4-48 months (mean 14.2 months) and duration of follow up time since the onset of eye symptoms ranged from 8 months to 12 years (Table 4). Twenty-four patients with Graves disease with ophthalmopathy (GO) (n=13) and without ophthalmopathy (n=12) and 12 euthyroid relatives from WH-Fam with a high prevalence of thyroid autoimmunity [13] were also studied. The severity and activity scores of the ophthalmopathy in patients with GO were slightly higher than in the patients with EGD (results not shown).

A positive Thyretain-TSI test was taken as SRR% > 140%, established in preliminary studies by Lytton [10-12]. The focus of the present study is the measurement of TSH-R antibodies in a Thyretain-TSI reporter bioassay in patients with EGD.

We carried out TSI tests on 39 blood samples from 12 patients with EGD. Tests were negative in 36 samples and positive in 3 patients at the time they converted to Graves' hyperthyroidism. TSI was measured early (< 6 months since the onset of eye symptoms) in 5 patients and later (> 6 months after the onset of symptoms) in the other 7 patients. Thyretain-TSI tests were always negative in 9 patients with EGD but positive, at one test only, in 3 patients (nos. 1, 2 and 3, Table 4). These 3 patients (nos. 1, 2, 3) are described in more detail; patient no. 1 was referred as "EGD" for eye muscle antibody testing and had a strongly positive Thyretain-TSI test; however, it turned out that she had developed Graves hyperthyroidism soon after the test. Patient no. 2 had been managed by his ophthalmologist and endocrinologist for many years as "EGD" and also had a strongly positive test in his first serum sample (an earlier TRAb test had been negative), just before he too converted to Graves hyperthyroidism. His Thyretain-TSI tested negative 4 months later (Table 4). Patient no. 3 had a 4 months history of "EGD" and a negative Thyretain-TSI test at the time of her first TSI test. Her second TSI test, 6 months later, was strongly positive and corresponded with the development of Graves hyperthyroidism soon after. She was then treated with ^{113}I, becoming hypothyroid at the time of her third, negative, test another 6 months later (Table 4).

All other tests in the other 9 patients were always negative and none of these patients have developed thyroid autoimmunity during follow up of from 6 months to 12 years. Thyretain-TSI levels at the first blood test only and mean (+/- SD) for the group are shown in Figure 2. Also shown in Figure 2 are initial Thyretain-TSI levels in patients with Graves' hyperthyroidism, GO, euthyroid members of WH-Fam and normal subjects. Tests were negative in all euthyroid relatives in WH-Fam and in all normal's (Figure 2). The mean (+/- SD) SRR% for patients with EGD, 95.2 (+/- 114) was just significantly different from that for the normal's [31.8 (+/- 35) SRR%, Mann –Whitney test, p = 0.02] because of the two patients with positive

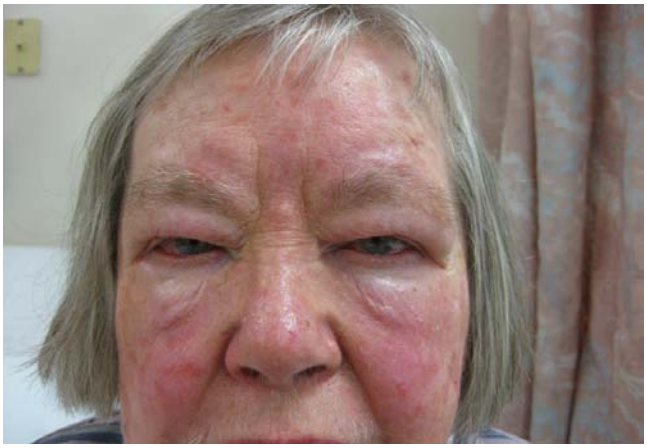

Figure 1: Patient with euthyroid Graves disease and mainly congestive changes; severe eyelid swelling, watery eyes, conjunctival injection and chemosis, but no exophthalmus and normal eye muscle function.

Table 4: Serum TSI by cAMP assay and TSI-Thyretain levels in 12 patients with Euthyroid Graves' disease, at each clinic visit.

Patient Number	Duration (months): ophthalmopathy onset to first TSI test	Serum [1]TSI-Thyretain level (SRR %)
1	4	**357%**
2	48	**332%**
	52	138%
3	4	90%
	10	**405%**
	16	67%
4	5	108%
5	18	43%
6	24	60%
	40	66%
	60	42%
	66	31%
	72	35%
	78	52%
	84	48%
	90	58%
	96	91%
7	30	44%
	32	45%
	46	50%
	51	42%
	60	44%
8	12	49%
	13	64%
	15	107%
	21	85%
	27	48%
	33	75%
	39	25%
	45	68%
	51	51%
	57	57%
	60	68%
	66	49%
9	18	43%
10	8	33%
11	5	43%
	8	44%
12	3	75%

[1] Measured as SRR% in a thyroid stimulating immunoglobulin (TSI) Reporter bioassay; a positive test (bold) is taken as an SSR% > 140%

tests at the first visit (nos. 1, 2 Table 4), who had converted to Graves' hyperthyroidism at this time. The other positive patient (no 3) had a negative test at the first visit and this value is not shown in Figure 2. Mean (+/- SD) SRR% for patients with GO (270 +/- 113 SRR%), and Graves hyperthyroidism without ophthalmopathy (228 +/- 94 SRR%] were both significantly increased compared to that for normal's ($p < 0.001$, $p < 0.001$, respectively). The difference between GO and Graves hyperthyroidism without ophthalmopathy was not significantly different (p = NS). While TSI levels tended to be higher in those patients with more severe or active eye disease, this was not significant (results not shown). Mean (+/- SD) SRR% for the EGD group was significantly greater than that for the normal's, mainly because of the two positive tests ($p = .0021$). Mean (48 +/- 19 SRR%) for the WH-Fam group was also significantly greater than that for the normal's ($p = 0.02$) but TSI tests were negative in all patients. We did not study the relationship between Thyretain-TSI levels and various parameters of the severity and activity (CAS, NOSPECS classes, Nunery class) of the eye disease.

Discussion

GO is a complex disorder because one must explain the unique link between the orbital inflammation and thyroid autoimmunity. One commonly held view is that the TSH-R antibodies that cause Graves' hyperthyroidism also cause the ophthalmopathy by cross-reacting with the TSH-R in the orbital connective tissue [3-6,17]. It is true that TSH-R antibody levels tend to be higher in patients with ophthalmopathy and very high in those with severe active disease [18] and that the hyperthyroidism and eye signs tend to occur together when TSH-R antibody levels are first detected [19]. However, there are some situations where these antibodies are not closely associated with ophthalmopathy and the overall evidence is mainly circumstantial [7]. One way to address a possible role of TSH-R antibodies in the development of "endocrine" or "autoimmune" ophthalmopathy is to study patients with so-called "euthyroid Graves' disease (EGD)", who have the same eye disorder but with normal thyroid function and no evidence for thyroid autoimmunity on long term follow up. If they become hyperthyroid – as did 3 of our patients - they are no longer considered to have EGD, but Graves' disease. We have measured TSH-R antibodies using a new and sensitive bioassay, which is closely linked to ophthalmopathy in patients with Graves' disease, the Thyretain-TSI reporter bioassay. To summarise the main results; Thyretain-TSI tests were strongly positive in the great majority of patients with Graves hyperthyroidism and GO, as expected, but negative in all normal subjects and in all euthyroid patients from a family with a high prevalence of ophthalmopathy and thyroid autoimmunity. Thyretain-TSI tests were also positive in 3 out of 12 patients with EGD, but all 3 had developed Graves' hyperthyroidism at or soon after the positive test and tests were negative in the other 10 patients at all clinic visits over a follow up period of up to 12 years.

The notion of classes of TSH-R antibodies with different actions namely, stimulating, binding and blocking, some of which may be implicated in the pathogenesis of the ophthalmopathy associated with Graves hyperthyroidism, and others the cause of the hyperthyroidism, is interesting and the focus of intense study. For example, one group [20] has claimed that only blocking TSH-R antibodies are linked to the ophthalmopathy of Graves' disease [21]. TSH-R blocking antibodies are presumed to be different from the stimulating antibodies measured in older cAMP driven assays and the TSI measured here in the Thyretain - TSI reporter bioassay. Lytton *et al* [10-12] showed that antibodies detected in the Thyretain-TSI reporter assay were closely linked to the ophthalmopathy in patients with Graves' disease whereas binding TSH-R antibodies were associated with the hyperthyroidism. We did not show a close relationship between positive Thyretain-TSI and ophthalmopathy in our patients with Graves' disease, but this may reflect the fact that the patients were unselected for duration and severity of the hyperthyroidism or severity or activity of any associated ophthalmopathy and most patients, with or without ophthalmopathy, had high Thyretain-TSI levels.

The key finding from our study is that Thyretain-TSI tests were always negative in patients with EGD unless they had converted to Graves' hyperthyroidism. In our series, only 3 patients have so far converted after up to 12 years follow up and it is not clear whether any others will develop Graves' disease in the future. In other words, the ophthalmopathy and hyperthyroidism appear to be quite separate organ specific autoimmune disorders, TSI as measured in the Thyretain ™ -reporter bioassay, being associated with hyperthyroidism, but not ophthalmopathy in patients initially diagnosed with "euthyroid Graves disease". However, we do need to measure TSH-R blocking antibodies in our patients with EGD, even though it seems unlikely that these antibodies would be detected when Thyretain-TSI and TRAb tests are only positive in those patients who have developed Graves' hyperthyroidism.

In conclusion, the finding of positive Thyretain -TSI tests in patients with Graves hyperthyroidism and GO, but not in patients with EGD until they developed Graves hyperthyroidism (which occurred in only 3 of our 12 patients studied for up to 12 years) suggests that TSH-R antibodies, regardless of how they are measured, may not play a major role in the pathogenesis of what is best called "autoimmune" or "endocrine" ophthalmopathy [22], although this needs to be confirmed in a larger, long term, study in which the various TSH-R antibody tests including TSH-R blocking antibodies are carried out. A pathogenic role of other antibodies in ophthalmopathy associated or not with Graves hyperthyroidism, such as those targeting collagen

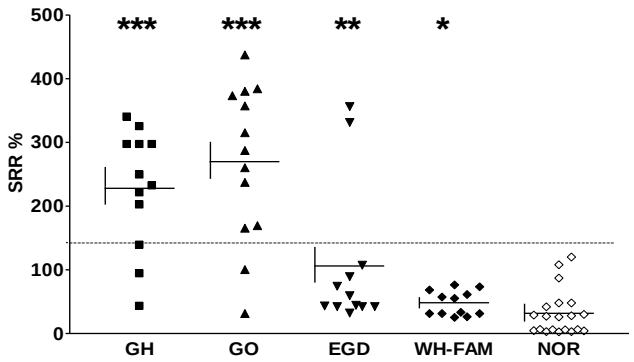

Figure 2: Individual Thyroid stimulating immunoglobulin (TSI) levels in patients with Graves hyperthyroidism (GH) with ophthalmopathy (GO, n=13), GH without ophthalmopathy (GH, n=12,) euthyroid Graves disease (EGD, n=12), euthyroid relatives from a single family having high prevalence of Graves disease and ophthalmopathy (WH-Fam, n=12) and normal subjects, as controls (NOR, n=20) and mean (+/- SD) for the groups. The manufacturers cut-off of positive TSI, SSR% = 140% is indicated by the dotted line. *** $p < .0001$, ** $p = .0021$, * $p = .0252$, as determined using Mann Whitney tests.

XIII, calsequestrin [23,24] or the 64-kDa protein [25], should now be taken more seriously.

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